HEMATOLOGY OF THE MONKE (MACACA MULATTA)

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ABSTRACT

Base line hematological values were determined for 79 male and 62 female monkeys (Macaca mulatta). One group of 5 males and 6 females over 3 years of age was sampled weekly for 40 weeks. A second group of 130 monkeys was subdivided according to sex and age: males vs females, under 3 years of age vs over 3 years of age. These animals were sampled biweekly for 12 weeks. Age- and sex-related influences on the various parameters were evaluated.

In the first group, the males' red cell count, hemoglobin, and hematocrit were significantly higher ($p < .001$) than the corresponding values for females. In females the white cell count, platelet count and erythrocyte sedimentation rate were significantly higher ($.05 > p > .01$) than in males.

In the second group, sex-related differences were seen in both age categories. Males under 3 years had significantly higher ($p < .01$) hemoglobin, hematocrit, lymphocyte and clotting time values than females of the same age group. In females under 3 years, the segmented neutrophil value was higher ($p < .01$) than in males under 3 years. In the animals over 3 years of age, males had higher values for red cell count, hemoglobin, hematocrit, lymphocyte, clotting time (all $p < .01$) and white cell count ($.05 > p > .01$). As in the younger age group, the segmented neutrophil value was higher ($p < .01$) in females.

Three age-related differences were found. Males over 3 years had a higher red cell count ($.05 > p > .01$) but a lower hemoglobin ($p < .01$) than males under 3 years. Females under 3 years had a higher white cell count ($p < .01$) than the older females.

The results emphasize the requirement for base line determinations and for assessment of the role of endogenous factors (e.g., age and sex) on the parameters of interest.

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I. INTRODUCTION

The Armed Forces Radiobiology Research Institute (AFRRI) has conducted extensive studies on the effects of different types of ionizing radiations in various mammalian species. Detailed hematologic analysis was an integral part of many of these studies. In addition to providing a valuable aid in determining the animal's suitability for definitive research, it affords a readily accessible means of obtaining an indication of the radiation effects on various mammalian tissues whose functions are reflected in the formed elements of the peripheral circulation.

The monkey (Macaca mulatta) has been the experimental animal of choice in many of the AFRRI studies. Although this animal has been used extensively in research, adequate information regarding its "normal" hematology is lacking in the literature. With one exception,[4] reported hematological values are based on a few observations obtained from a small number of animals. Krise[4] utilized a large number of male monkeys (535 Macaca mulatta) in one short-term study with apparently two or less observations being taken on each animal. In a more recent study by the same investigator,[3] many observations were taken on 12 male animals. While both studies evidently satisfied their desired objectives, they did not provide the data required for the AFRRI investigations since they were accomplished on males only.

The objectives of the work reported herein were as follows:

a. To determine "normal" hematological values for a frequently used research animal.

b. To establish base line values for animals intended for subsequent radiation effects studies.

c. To evaluate age- and sex-related influences on the parameters considered.

II. MATERIALS AND METHODS

The 79 male and 62 female Macaca mulatta used in this study were "wild-caught" animals imported from the highlands of northern India.* The animals were subjected to a 30-day conditioning period prior to shipment to AFRRI and a 42-day conditioning period subsequent to their arrival. Tuberculin testing, deworming, and indicated specific therapy were performed during these periods. Animals were housed individually in Harford primate cages.† The monkeys were fed three times each day. Their basic diet consisted of Purina Monkey Chow supplemented on alternate days with fruit (1/4 apple or 1/4 orange) or vegetable (1 kale leaf) following the morning feeding.

Blood samples were collected prior to the morning feeding. Each sample consisted of approximately 5 ml of blood removed via femoral venipuncture utilizing a 5 ml plastic syringe fitted with a 1-inch 21 gauge needle. Four ml of blood were transferred to a plastic vial containing approximately 8 mg of the dipotassium salt of ethylenediaminetetraacetic acid (EDTAP) as an anticoagulant. The remaining 1 ml which was to be used for clotting time measurements and serum collection was placed in a glass vial containing no anticoagulant.

The first series of observations included 5 males and 6 females sampled weekly for 40 consecutive weeks. These animals are identified in this report as

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* Asiatic Animal Imports, Inc., San Francisco, California.

† Model No. SM 2020-S, Harford Metal Products, Inc., Aberdeen, Maryland.

Group I. At the beginning of the study, Group I monkeys weighed from 4.5 to 6 kg and were 3.5 to 6 years of age (as determined primarily by dentition[2] and correlated with weight and crown-to-rump measurements[8]).

The second series of observations included 74 males and 56 females sampled every other week for 12 weeks. These animals, identified in this report as Group II, were further divided according to age:

36 males under 3 years old	(age range: 24-32 months)
38 males over 3 years old	(age range: 36-60 months)
22 females under 3 years old	(age range: 24-32 months)
34 females over 3 years old	(age range: 36-60 months)

Three years of age seems to approximate the time of sexual maturity for the Macaca mulatta.[6]

The blood parameters were measured using the following described hematological procedures which are further amplified by Wintrobe,[9] Schalm,[5] and Annino[1]:

Red blood cell count. Blood was diluted 1:50,000 with 0.9 percent NaCl solution prior to counting with an electronic cell counter.* Counts were corrected for coincidence losses.

White blood cell count. Blood was diluted 1:500 with 0.9 percent NaCl solution and sufficient 1 percent saponin solution (2 drops, or approximately 100 μl) was added to obtain a saponin concentration of 1:10,000. After a lapse of 25 minutes to permit lysis of the red cells, counts were performed using the electronic cell counter.

* Model B, Coulter Electronics, Hialeah, Florida.

Hemoglobin. Blood was diluted 1:250 with Drabkin's solution. The optical density of the resulting cyanomethemoglobin was measured at 540 nm using a Coleman Junior spectrophotometer.* The optical density was converted to hemoglobin concentration using a previously constructed standard curve.

Microhematocrit. Blood was drawn into a capillary tube and the tube was sealed at one end. The hematocrits were read after the tubes had been centrifuged for 5 minutes at 10,000 rpm in a microhematocrit centrifuge.+

Reticulocyte counts. A drop of blood was mixed with a drop of new methylene blue stain. Twenty minutes later a smear was prepared and the number of reticulated red cells per 1000 red cells was counted.

Differential white blood cell counts. Blood smears were stained with Wright-Giemsa stain and 100 white cells were differentiated.

Platelet counts. Blood was diluted 1:100 with a 1 percent ammonium oxalate solution containing 0.5 percent formalin. After mixing the blood and diluent for 10 minutes, a hemocytometer was charged and allowed to stand in a moist atmosphere for 20 minutes. The platelets in 0.2 mm^2 were enumerated using a phase microscope.

Erythrocyte sedimentation rate. A Wintrobe hematocrit tube was filled with blood and placed in a vertical position. The number of millimeters the red cells had settled in 1 hour was recorded.

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* Coleman Instrument Company, Maywood, Illinois.

+ Clay-Adams, Inc., New York, N. Y.

Clotting time. The clotting time was measured using the Lee-White one-tube method. Clotting time was computed to the nearest half-minute starting with the time of flow from the vein and extending until blood flow had ceased as determined by periodic partial inversions (approximately 90^{0}) of the containing glass vial.

Total serum protein. The total protein content of the serum was measured using the biuret method.

Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from the mean values of the red blood cell count, hematocrit, and hemoglobin.

III. RESULTS

Group I: Forty weekly bleedings of 11 monkeys.

The results obtained by bleeding 11 Macaca mulatta weekly for 40 weeks are summarized in Table I. Table II contains the same data differentiated according to the sex of the animals (5 males and 6 females).

Table I. Hematologic Values for Adult Male and Female Macaca mulatta*

Parameter	Units	Number of observations	Mean ± Standard Deviation	Range
red blood cell count	cells/mm^3 x 10^6	432	5.31 ± 0.47	3.76 - 7.56
white blood cell count	cells/mm^3	432	8639 ± 2456	3638 - 19,968
hemoglobin	g/100 ml	432	12.7 ± 1.3	7.6 - 17.2
hematocrit	volume percent	432	39.9 ± 3.6	25 - 49
reticulocyte count	percent of RBC's	426	0.99 ± 0.39	0.1 - 2.5
segmented neutrophils	percent of WBC's	432	37.2 ± 13.0	4 - 79
lymphocytes	percent of WBC's	432	56.9 ± 12.8	19 - 91
platelet count	cells/mm^3 x 10^3	399	517.6 ± 102.2	260 - 920
sedimentation rate (uncorrected)	mm/h	432	0.49 ± 1.4	0 - 10
clotting time	minutes	374	6.0 ± 1.5	2.5 - 13.5
total protein	g/100 ml serum	434	7.9 ± 0.5	6.7 - 10.4
MCV†	μ^3	-	75.5	-
MCH†	$\mu\mu$g	-	24.1	-
MCHC†	volume percent	-	31.9	-

* Based on 40 weekly bleedings of 5 male and 6 female Macaca mulatta, age 3-1/2 - 6 years.

† Calculated from weekly means of red cell count, hematocrit and hemoglobin.

Table II. Sex-Related Comparisons in Macaca mulatta Hematology*

Parameter	Units	Males		Females	
		Number of observations	Mean ± Standard Deviation	Number of observations	Mean ± Standard Deviation
red blood cell count	cells/mm^3 x 10^6	129	5.54 ± 0.43[1]	233	5.10 ± 0.39[1]
white blood cell count	cells/mm^3	199	8221 ± 2358[2]	233	8938 ± 2479[2]
hemoglobin	g/100 ml	199	13.2 ± 1.3[1]	233	12.3 ± 1.3[1]
hematocrit	volume percent	199	41.3 ± 3.5[1]	233	38.7 ± 3.4[1]
reticulocyte count	percent of RBC's	198	0.91 ± 0.39	230	0.87 ± 0.35
segmented neutrophils	percent of WBC's	199	37.6 ± 14.4	233	36.9 ± 11.8
lymphocytes	percent of WBC's	199	56.3 ± 14.0	233	57.9 ± 11.4
platelet count	cells/mm^3 x 10^3	184	496.4 ± 85.7[2]	215	535.7 ± 103.4[2]
sedimentation rate (uncorrected)	mm/h	199	0.4 ± 0.8[2]	233	0.5 ± 1.2[2]
clotting time	minutes	170	6.0 ± 1.5	204	6.5 ± 1.5
total protein	g/100 ml serum	200	7.9 ± 0.4	234	7.8 ± 0.6
MCV*	μ^3	-	74.8	-	76.3
MCH*	μg	-	23.7	-	24.3
MCHC*	volume percent	-	32.0	-	31.9

* Based on 40 weekly bleedings of 5 male and 6 female Macaca mulatta, age 3-1/2 - 6 years.

* Calculated from weekly means of red cell count, hematocrit and hemoglobin.

[1] $p < .001$ [2] $.05 > p > .01$

Comparisons made using Student's t-test revealed highly significant ($p < .001$) differences in red cell count, hemoglobin, and hematocrit which were apparently related to sex. The higher values were found in the males. Also, significant differences ($.05 > p > .01$) were seen in white cell counts, platelet counts, and erythrocyte sedimentation rate. These parameters were seen as higher values in the females.

Group II: Six biweekly bleedings of 130 monkeys.

Table III summarizes results obtained from the Group II observations (74 male and 56 female Macaca mulatta).

These data were compared using analysis of variance. When this test revealed the existence of a difference significant at the 5 percent level, individual means were compared using Duncan's multiple range test modified for unequally replicated

Table III. Sex- and Age-Related Comparisons in Macaca mulatta Hematology *

Parameter	Units	♂ under 3 yrs		♀ under 3 yrs		♂ over 3 yrs		♀ over 3 yrs	
		n†	Mean ± S.D.‡	n†	Mean ± S.D.‡	n†	Mean ± S.D.‡	n†	Mean ± S.D.‡
red blood cell count	cells/mm^3 x 10^6	211	5.70 ± 0.59	127	5.57 ± 0.55	219	5.85 ± 0.62	201	5.60 ± 0.57
white blood cell count	cells/mm^3	212	7885 ± 2351	127	8508 ± 3182	219	8200 ± 3216	201	7572 ± 2373
hemoglobin	g/100 ml	212	14.1 ± 0.9	127	13.4 ± 1.1	219	13.8 ± 1.0	201	13.5 ± 1.0
hematocrit	volume percent	212	41.9 ± 2.6	127	40.9 ± 3.0	219	42.1 ± 2.8	201	41.1 ± 2.8
reticulocyte count	percent of RBC's	212	0.59 ± 0.45	126	0.72 ± 0.48	219	0.68 ± 0.46	201	0.68 ± 0.45
segmented neutrophils	percent of WBC's	213	32.5 ± 13.5	125	37.0 ± 15.3	219	34.5 ± 14.3	201	36.7 ± 13.0
lymphocytes	percent of WBC's	212	63.8 ± 13.3	125	57.8 ± 14.3	219	61.3 ± 14.3	201	56.7 ± 12.3
platelet count	cells/mm^3 x 10^3	187	560.7 ± 174.0	113	558.8 ± 173.7	196	581.8 ± 165.3	162	574.3 ± 152.7
clotting time	minutes	210	6.0 ± 1.5	127	5.5 ± 1.0	215	6.0 ± 1.5	197	5.5 ± 1.0
total protein	g/100 ml serum	211	8.3 ± 0.7	128	8.3 ± 0.6	219	8.4 ± 0.7	200	8.2 ± 0.8
MCV§	μ^3	-	74	-	73	-	72	-	74
MCH§	μg	-	25	-	24	-	24	-	24
MCHC§	volume percent	-	33.6	-	32.9	-	32.8	-	33.0

* Based on 6 biweekly bleedings of 22 females and 36 males under 3 years of age and 34 females and 38 males over 3 years of age.

† n = number of observations.

‡ S.D. = Standard Deviation.

§ Calculated from biweekly means of red cell count, hematocrit and hemoglobin.

treatments.[7] Table IV lists the parameters for which meaningful significant differences were found.

The highly significant sex-related differences in the red cell count, hematocrit, and hemoglobin seen in the Group II animals were evidenced at the $p < .01$ significance level in the Group II animals over 3 years of age. With the exception of the red cell count parameter, these differences were also found in the Group II animals less than 3 years of age. In all cases where significant sex-related differences were found in the red cell count, hemoglobin, and hematocrit, the male values exceeded the female values.

Sex-related differences in other parameters were also seen in the Group II animals. These differences, found in both age groups, included the segmented neutrophils, lymphocytes, and clotting time. Higher segmented neutrophil values in both

age groups were observed in the females, whereas the males in both age groups exhibited the greater lymphocyte and clotting time values. Differences in these three parameters were not seen in the Group I animals.

Table IV. Statistically Significant Sex- and Age-Related Differences in Macaca mulatta (Group II) Hematology*

Comparison	Parameter	Larger value	Result of significance test
males vs. females (under 3 years)	hemoglobin	males	$p < .01$
	hematocrit	males	$p < .01$
	segmented neutrophils	females	$p < .01$
	lymphocytes	males	$p < .01$
	clotting time	males	$p < .01$
males vs. females (over 3 years)	red cell count	males	$p < .01$
	white cell count	males	$.05 > p > .01$
	hemoglobin	males	$p < .01$
	hematocrit	males	$p < .01$
	segmented neutrophils	females	$p < .01$
	lymphocytes	males	$p < .01$
	clotting time	males	$p < .01$
under 3 years vs. over 3 years (males)	red cell count	over 3 years	$.05 > p > .01$
	hemoglobin	under 3 years	$p < .01$
under 3 years vs. over 3 years (females)	white cell count	under 3 years	$p < .01$

* Significance tests on data presented in Table III. A modified Duncan's multiple range test was used on groups of means for which a significant ($p < .05$) F-value was found upon analysis of variance.[7]

A sex-related difference in white blood cell count was seen only in the older age component of Group II and this was at the level of $.05 > p > .01$. This finding was not consistent with the Group I animals whose ages were comparable to the over 3 years of age component of the Group II animals in that the male white blood cell values exceeded the female in the latter and the inverse was the case in Group I.

Age-related differences in two parameters (red cell count and hemoglobin) were observed in the male animals. The higher red cell counts ($.05 > p > .01$) were found in the male animals over 3 years of age, but the higher hemoglobin values ($p < .01$) were found in the male animals under 3 years of age.

An age-related difference was noted in only one parameter (white cell count) in the female animals. In this instance, the higher values ($p < .01$) were found in the animals under 3 years of age.

IV. DISCUSSION

The base line hematology of the Macaca mulatta as determined by this laboratory is presented as a reference for future comparisons. These data do not alter the requirement for experimental controls. However, base lines are useful in such instances as:

a. Assessment of the accuracy of a laboratory procedure.

b. Determination of the effect of environmental changes or disturbances.

c. Evaluation of fluctuations in control values.

d. An aid in determining an animal's suitability for experimentation.

In biological investigations involving such obvious sex- and age-related variables as endocrine function and tissue differentiation, the sex and age of the experimental animal are major considerations. With other variables, e.g., hematology, an existing relationship to sex and age may be less striking. However, these less obvious sex- and age-related differences should be considered in the experimental design and in the evaluation of the resultant data.

Most of the hematological values presented in this report fall within the ranges for this animal as reported by Krise.[3,4] The major exception is clotting time. Although the method of determination employed in this study was apparently similar to the one used by Krise, the clotting times of the AFRRI monkeys were much longer

(approximately 6 minutes for the AFRRI animals as compared to 1 minute 55 seconds for the Krise animals).

Variation among individuals is a common feature of a relatively heterogeneous group of animals such as the Macaca mulatta. Thus, the ranges and standard deviations of most of the hematologic parameters considered here were large, as has been the case in other studies.[3] This fact serves to emphasize the requirement for repeated observations on a large number of animals before such data can be considered to be a reliable reflection of base line values.

An interesting observation made during the conduct of this study was the confirmation of the value of the erythrocyte sedimentation rate as a diagnostic tool. Without exception, monkeys whose sedimentation rate exceeded 5 mm/h exhibited clinically detectable inflammatory conditions or transient physiological alterations. Throughout the course of the study, elevated sedimentation rates were obtained on nineteen animals. While the majority were in females and associated with menstrual periods, four elevated rates were obtained on apparently healthy animals. The results of a thorough examination indicated the existence of a severe respiratory infection in all four animals. These animals were immediately removed from the study and the tentative diagnosis was ultimately confirmed.

Perhaps the most important conclusion to be drawn from this study is that extensive base line determinations should be performed prior to using a wild animal in biological experiments, due to the inherently large variation in such a population. However, data obtained on a conditioned animal, clinically healthy, and maintained under specified laboratory conditions are probably the best possible for a wild-caught

species. As demonstrated by this study, it is also necessary to assess the role of other endogenous influences, such as age and sex, on the values obtained. Published base lines obtained in other laboratories are valuable aids. However, they need to be supplemented by an investigator's own confirmatory analyses to provide reliable biological data for definitive animal experimentation.

V. SUMMARY

Base line hematology results are presented for 141 male and female monkeys (Macaca mulatta). Significant differences which appear directly related to sex and age are reported. The results emphasize the requirement for base line determinations and for the assessment of the role of endogenous influences (e.g., age and sex) on the parameters of interest.

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